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**Towards an integrated understanding of how micro scale processes shape groundwater ecosystem functions**

Short title: Micro scale processes shape groundwater ecosystem functions

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**Highlights**
- The micro scale shapes the groundwater food web
- The implications of the micro scale in biodegradation remain to be clarified
- So far, lack of sampling methods at appropriate scales led to mixed samples
- Groundwater fauna may be used as a biomanipulating tool in bioremediation

**Abstract**

Micro scale processes are expected to have a fundamental role in shaping groundwater ecosystems and yet they remain poorly understood and under-researched. In part, this is due to the fact that sampling is rarely carried out at the scale at which microorganisms, and their grazers and predators, function and thus we lack essential information. While set within a larger scale framework in terms of geochemical features, supply with energy and nutrients, and exchange intensity and dynamics, the micro scale adds variability, by providing heterogeneous zones at the micro scale which enable a wider range of redox reactions. Here we outline how understanding micro scale processes better may lead to improved appreciation of the range of ecosystems functions taking place at all scales. Such processes are relied upon in bioremediation and we demonstrate that ecosystem modelling as well as engineering measures have to take into account, and use, understanding at the micro scale. We discuss the importance of integrating faunal processes and computational appraisals in research, in order to continue to secure sustainable water resources from groundwater.

# 1    Introduction

Scientists and engineers approach groundwater systems regularly on large scales of km (Fitts, 2012) and make their assumptions regarding e.g. protection zones for drinking water production and regarding bioremediation on these scales (e.g. by deriving parameters for the Darcy equation applied to the whole aquifers based on point measurements of aquifer properties, Wendland et al., 2004). In contrast, bioremediation measures such as pump-and-treat or reactive walls are installed on the m scale. Usually, these approaches are helpful and often successful in reaching the aims demanded by guidelines such as the European Commission nitrate directive 91/676/EEC or the European Commission water framework directive 2000/60/EC with the groundwater directive COM(2003)550, but here we want to discuss how much more we can learn and achieve, if we include the micro scale processes in the large-scale considerations. Bertrand et al. (2014) demonstrated the usefulness of micro scale investigations for the hyporheic zone, and it is reasonable to hypothesise that a focus on the micro scale will be as informative in groundwater ecosystem s more broadly.

In the context of the present discussion, by 'micro scale' we mean scales of less than one millimetre. Groundwater bacteria, fungi, and archaea (archaea being a kingdom of unicellular organisms lacking a nucleus and membrane-bound organelles, like bacteria, but harbouring physiological and genetic features very different from bacteria; Fox et al., 1980) are regularly smaller than 0.001 mm (Griebler et al., 2002). Among the fauna, worms, rotifers, and micro arthropods are up to 1 mm, and the largest groundwater arthropods are usually around 10 mm (Wilkens et al., 2000). The size of protozoa, i.e. unicellular animals, ranges in-between that of multicellular organisms and bacteria, fungi, and archaea. For the purpose of this paper, everything above the micro scale is considered as meso or macro scale – terms which are defined variably in the literature - and we don't seek to redefine them here since this discussion is solely concerned with stressing the micro scale importance. This is not to deny that there is considerable heterogeneity on the meso & macro scales having implications on the whole system. E.g. a low permeability patch on the stream surface, over scales of several meters, behaves differently depending on the permeability of the sediment surrounding it (Ward et al., 2011). Similar patterns have been shown to occur in groundwater sediments (e.g. Schmidt et al., 2007). However, this larger scale heterogeneity has been discussed in the context of "hot spots-hot moments" (McClain et al., 2003), or "beads on a string" (Stanford and Ward, 1993) along rivers, pools and riffles (Wiens, 2002) extensively already, also for the interface between rivers and groundwaters (Schmidt et al., 2007b). Here, we want to focus on the groundwater micro scale ecosystem heterogeneity and its implications for biological processes. To do this we have to differentiate processes on larger meso and macro scales (discussed below) from those on the micro scale (section 2).

The macro scale (> 1 km) drives food webs and ecosystems in that it shapes the general context. For example, the geological setting is a primary control on the hydrochemical conditions. The total ion content, and thus hardness, depends directly on the solubility of the mineral matrix. The stratigraphy of geological units, in combination with soil properties and climate, determines recharge patterns and thus where the water in an aquifer is coming from. If recharge and thus exchange are strong, there is a good chance for provision of allochthonic (foreign to the system)

input, and, most importantly, dissolved oxygen. In contrast, in a secluded part of the aquifer, replenishment with dissolved oxygen, oxygenated compounds or other resources is likely to be low and thus, life has to adapt to limited available energy from resources. Combined with prevailing land use patterns, larger scale recharge distributions govern the extent to which anthropogenic inputs may be introduced into aquifers (e.g. contamination; see section 3.2). Spatial variability in groundwater recharge may occur at scales ranging from cm to km (Fig. 6 of Cuthbert, 2014).

Fauna, i.e. unicellular protozoa, worms, crustacea, and basically all other phyla known from the surface, as well as bacteria, archaea, and fungi have been found in all types of groundwater, regardless of geological or climatic setting or redox situation (Botosaneanu, 1986; Boulton et al., 2008; Gibert et al., 1994; Griebler and Lueders, 2009; Hakenkamp and Palmer, 2000; Wilkens et al., 2000). Protozoa, i.e. unicellular animals, are sometimes included in the rather loose term microorganisms due to their small size and their organisation within one cell, but in terms of genetic, biochemical, physiological, cytological, and developmental features, as well as feeding modes, they belong to fauna which comprises the unicellular protozoa and the multicellular metazoa.

Groundwater organisms have developed morphological and physiological adaptations to this special environment (Coineau, 2000). Microorganisms only seem to be restricted by temperatures clearly exceeding 120°C unless temporarily (Clarke, 2014; Cowan, 2004). Metazoa are more restricted; but neither depth (1000 m in Marocco; Essafi et al., 1998; depths of 800 m in the Texan St. Edwards aquifer; Longley, 1992; nematods in 1300 m depth in South African gold mines: Borgonie et al., 2011), nor pore size distribution (Schmidt et al., 2007a) or low oxygen values (Galassi et al., 2016; Malard and Hervant, 1999; Por, 2007; Riess et al., 1999), necessarily exclude fauna from a groundwater zone – the patterns are complex. Groundwater metazoa are partly more sensitive towards contaminants, partly less sensitive than their closest relatives on the surface and might survive under conditions that their surface relatives experience as lethal. E.g. the stygobitic (i.e. home to groundwater) *Crangonyx pseudogracilis* proved more sensitive to chromium than the stygoxene (i.e. foreign to groundwater; only invading occasionally) *Gammarus fossarum* (Canivet et al., 2001). The opposite pattern was observed e.g. for the North American stygoxene *Gammarus minus* which was more sensitive towards toluene than the Middle European stygobite *Niphargus inopinatus* (Avramov et al., 2013). One adaptation is a high motility which leads to distributions which are patchy in time and space (Brancelj and Dumont, 2007; Hancock and Boulton, 2008; Kasahara et al., 2009).

While there is a huge body of knowledge on macro and mesoscale groundwater ecology, discussion, let alone data on how the micro scale microbial processes might influence the whole food web is lacking from all these reviews. Particularly for groundwater, growth rates of all organisms, degradation rates, reproduction rates, and feeding rates are still seriously understudied and remain largely unknown.

After this general introduction into the groundwater ecosystem, the (ecosystem) features that are most influential at the micro scale are described in section 2 and the implications of micro scale interactions for larger scales are discussed in section 3. This is followed by a section on practical applications (section 4), and rounded up with some conclusions. In all points, we restrict ourselves to unconsolidated

sediments, e.g. alluvial aquifers, in this contribution. This is not to deny the importance of crystalline aquifers (compare e.g. Johns et al., 2014) or karst (Goldscheider et al., 2006; Humphreys, 2006), but knowledge is still too patchy (Eisendle-Flöckner and Hilberg, 2015) on such aquifers to make generalized assumptions. General knowledge on unconsolidated sediments aquifers, in contrast, has been reviewed in Boulton et al. (1998); Boulton and Hancock (2006); Gibert et al. (1994); Griebler and Avramov (2015); Jones and Mulholland (2000); Schmidt and Hahn (2012); Wilkens et al. (2000), and, the most comprehensive compilation to-date, by Griebler and Mösslacher (2003a) as well as the focused recent volume by Brendelberger et al. (2015), the latter two in German though. In the following chapters we will only list those points most important for this discussion.

## 2   Which factors determine the micro scale ?

While groundwater ecosystems are already complex on the macro and meso scale, as outlined above, the complexity increases on the micro scale. The sources of the physical environment heterogeneity at the micro scale in groundwater ecosystems result from heterogeneity in grain size distributions, and from differences in shape of the matrix particles and their mineral composition. So called 'multiporosity' may result, whereby distinct modes in the pore size distribution (and therefore also in permeability) leads to preferential flow at one or more scales. Micro scale heterogeneity will also be the result of patchy bioreactions, as shown below and as shown for streams by Mendoza-Lera and Mutz (2013) or Harby et al. (2017).. This has consequences for larger scale patterns in groundwater as well, both in terms of the whole food web structure, but also in terms of overall productivity (similar to the upscaling of nitrogen uptake in surface stream sediments; Peipoch et al., 2016). It also has consequences for basic theoretical understanding and for practical applications, as explained in the following sections.

The micro scale is the scale on which microorganisms, which make up the highest proportion of biomass in presumably all groundwater ecosystems (Gibert et al., 1994), grow and (re)act. In groundwater sediments, bacteria and archaea are known to occur patchily in micro colonies of around 50 cells (Harvey et al., 1984; Iltis et al., 2011; Voisin et al., 2016), not continuous biofilms (except for zones in intense exchange with the surface, e.g. on groundwater pumps; Benedek et al., 2016), and this has bottom-up implications for the organisms feeding on the bacteria, fungi, and archaea, i.e. protozoa and metazoa (see section 3 for the general introduction to the groundwater food web).

Larger-body-sized biota such as crustacea move over larger distances than microorganisms and thus integrate over larger aquifer volumes and cover parts of the meso scale (Schmidt and Hahn, 2012), since they potentially use more different physical and chemical situations in a shorter period of time than shorter-range organisms. Thus they "see" more situations than could be connected by diffusion/advection in such systems and may act as mediators between the scales.

While the macro and meso scales set the general scene (see section 1), the timing, the range of types, and the number of biochemical reactions are decided on the micro

scale. The considerable micro scale variability provides micro-niches for the organisms, but may also cause constraints (Rebata-Landa and Santamarina, 2006). Two pores that are adjacent to each other and in general receive the same type of macro scale-influenced water, may differ in micro scale flow patterns due to the complexity of the micro scale hydraulics. As much as mineral distribution varies on the micro scale, sorption varies on the micro scale as well. This may lead to situations substantially different in terms of all sorts of physical and chemical properties (e.g. Figure 1; Briggs et al., 2015), and thus offer completely different habitats for organisms. One pore might be flown through, thus receiving a steady input of the macro scale-influenced water (bottom left half of Figure 1). Another pore might be relatively cut-off from any replenishment and receive input only on diffusive timescales, and may turn nutrient-deprived and its redox potential may decrease. This may be a permanent situation for example in the case of a dead end pore (top left and right in Figure 1), or a temporary condition in the case of a blockage to flow such as from a gas bubble, or microbial colonies (bottom right of Figure 1). A finely textured matrix is composed of small pores which may be blocked by even low numbers of cells (Nambi et al., 2003).

The diffusive timescales at which solutes equilibrate into 'cut-off' pores is related to the square of the characteristic pore length divided by the diffusivity. Thus, the geochemical spatial gradients at the micro scale will depend on the temporal variation in the macro scale input - the resulting 'output' to the hydraulically downstream organisms is therefore an integration of factors at these two scales.

Different niches are thus created in which different biochemical reactions are possible and take place. The reactions vary in the reaction partners, the activation energy required, the energy gained, as well as the reaction products, and might be manifold so that two situations directly adjacent to each other use completely different electron acceptors, electron donors, at completely different redox states and pH. Anaerobic microorganisms cannot thrive in oxic conditions, and can thus only be active in cut-off pores (dark-shaded zones in Figure 1). However, they might be able to use substances that will not, or even cannot, be used in oxic situations because they are not energetically favourable or possible as resource to aerobic organisms. Thus, occupants of such niches offer ecosystem services that would otherwise be missing from the system, and might close the gap in budgets and mass balances (e.g. Meckenstock et al., 2014). Heterogeneity in time and space is one reason why reduced iron may be found in an oxic aquifer - it is produced in pockets/patches and is then complexed by organics present and might therefore be found in considerable concentrations. Without knowledge on micro scale processes, the overall situation of the aquifer cannot be understood.

Once established and thriving, circumstances allowing, microbial cells shape the immediate surroundings on a scale of up to a few µm, by using resources such as dissolved oxygen or by exuding metabolic products, e.g. $CO_2$. (Note that for most metabolic products from one type of organism there is a type of microorganism which uses it – e.g. some autotrophs "feed" on $CO_2$ as their primary energy source). Such micro scale variation of resources will lead to the growth of the same physiological type being compromised, while other cell types might thrive. The out-competing leads to the majority of groundwater microbial cells being inactive, waiting for the resource

situation to improve (e.g. Weaver et al., 2015), but at the same time providing a wide range of biochemical reactions given the appropriate circumstances.

## 3 Interactions between producers, degraders, and predators

Food webs in general are driven bottom-up (e.g. by food availability), and top-down (e.g. by predation or virus mortality etc.). The producers, mostly bacteria, fungi and archaea, transform inorganic molecules and/or organic dead substrate (detritus) into biomass. Autotrophy, i.e. biomass production reliant on energy other than organics, exists in groundwater (Alfreider et al., 2003; Stevens, 1997). However, it is not driven by photic energy, but only chemical energy, and the extent of autotrophy remains largely unknown (Kellermann et al., 2011), although the importance for the whole food web in some systems has been stressed recently (Hutchins et al., 2016). The producers are grazed upon by consumers (protozoa and metazoa) which in turn are preyed upon by predators (metazoa). Predation, i.e. feeding on food web levels from the consumers onwards, seems to be negligible in groundwater ecosystems and food webs are therefore truncated (Gibert and Deharveng, 2002).

Most fauna will feed as generalists, switching between detritus, microbial colonies, and the occasional rare prey to a degree varying among taxa, but no groundwater organism will sustain its existence on prey alone – the dominant food source will be microbial colonies and detritus (Griebler and Mösslacher, 2003a; Marmonier et al., 1993). It is still appropriate to call it a food web even with "just" producers and consumers, because the microbial loop or microbial web (Azam et al., 1983; Fenchel, 1982) applies to groundwater as well (Griebler and Mösslacher, 2003b) – so there are consumers within the microbial loop and consumers of the second order grazing on the whole microbial loop. For instance, macroinvertebrates grazing biofilms will also consume ciliates feeding on biofilms, at least in streams (Lear et al., 2012). Thus, even without predators in the strict sense, the groundwater feeding relationships forms a complex web. The lack of predators does not necessarily mean reduced ecosystem services, since predator abundance and richness were both found to compromise multifunctionality and thus, ecosystem services (Soliveres et al., 2016) While the cited work focused on grasslands, we do not know how transferable this finding is to groundwater systems.

Biomanipulation, i.e. steering the relationship between trophic levels, has proven successful for lake ecosystem structure restoration under specific circumstances (Benndorf, 1995; Kasprzak et al., 2002), but has not been tested in groundwater management yet. However, it is undeniable that not only groundwater bacteria and archaea, but also groundwater fauna perform ecosystems services (Avramov et al., 2010) which are at the root for e.g. the sustainable production of drinking water. Our aim here is to show how patterns on the micro scale, in all organisms, influence larger scale patterns, similar to the upscaling in surface stream sediments (Peipoch et al., 2016), particularly when focusing on biodegradation.

One of the interactions taking place on the micro scale is the layering in microbial colonies such as biofilms are layered at the same scale in stream sediments (Battin et al., 2003). The layers consist of different functional groups each performing different biochemical reactions (e.g. Fig. 3a in Flemming et al., 2016). Such layering has rarely

been taken into account in any groundwater field investigation or any computational model, except for e.g. Beyenal and Lewandowski (2005); Kreft and Wimpenny (1998); Nadell et al. (2013). Layered microbial communities are used, however, for engineered systems where feedbacks, for example due to microbially induced precipitation reactions, may be created whereby access to resources or reactants is either diminished or increased by microbial activity (Cuthbert et al., 2012; Picioreanu et al., 2016). It is possible that further engineering approaches based on the spatially distributed organisation of microbial communities could be developed for further aims.

Fauna have to adapt to finding, and feeding on, these microbial growth types. Note that protozoan grazing on biofilms and micro colonies modify their architecture, as known from streams (Böhme et al., 2009), and thus create further heterogeneity on the micro scale. Knowledge on search behaviour, feeding preferences, as well as feeding rates is still too scarce for fauna in unconsolidated as well as fractured rock aquifers to make valid assumptions (Schmidt and Hahn, 2012).

## 3.1 Biotic interactions shape physical and chemical micro scale heterogeneity

Expanding from the general micro scale heterogeneity sketched in Figure 1, in Figure 2 a more complex picture of how micro scale features influence larger scales, and *vice versa*, is introduced. It combines the smaller scale details from Figure 1 with the dimensions of time (x axis in Figure 2) and space (y axis in Figure 2). The two figures have in common that exchange (with the surface) is one dominant driver of groundwater ecosystems on the micro scale (Figure 1), but also when combining all scales (Figure 2). The main point about Figure 2 is that situations which are very different in their physical, chemical, and hydraulic properties exist in close vicinity both spatially and temporally within the same macro scale framework and provide the environment for strikingly different biological communities and ecosystem functions which vice versa shape their physical and chemical environment. Even though the macro scale sets the scene for organism growth, the actual food web depends on the range of such micro scale environments in the pores, the resulting patchy microbial growth and the subsequent micro environments created, as well as the organisms' reaction to them. Sampling cannot yet reflect this productive micro scale variability appropriately (section 4.2).

The bottom-left micro scale pattern of reactions is the bottom-up basis for the microbial grazing on the meso level that forms the basis for the "fast" food web (Figure 2a-c). This sketches a comparably well-fed situation where a comparably high number of organisms finds enough resources to thrive, and thus grows in less time, i.e. on the smaller time scale, to larger (colony) sizes and diversity. Exemplary reactions on the shorter time scale (Figure 2a-c) might e.g. be the oxic degradation of labile acetate (substrate B in Figure 2c) under the usage of nitrate (substrate A in Figure 2c). $CO_2$ (substrate D in Figure 2c) is then produced which, depending on the circumstances, might form gas bubbles which may impact on flow patterns (Figure 2b; Cuthbert et al., 2010; Mendoza-Lera and Mutz, 2013).

The situation in Figure 2d–e, in contrast, stands for a much less-well fed situation where growth takes longer and, being slower, is more expensive in the sense of

activation energy and consequently produces less biomass and products. One example might be slow and biochemically "expensive" reactions such as the anoxic degradation of catechol (substrate F in Figure 2f), which is an intermediate compound in the benzene degradation pathway (Gibson et al., 1968) and for many organisms a potential inhibitor (Bergauer et al., 2005). Few reactions based on catechol are possible – one of them uses carbonate from the geological matrix as an electron acceptor (substrate E in Figure2f; Bennett et al., 2000). Only specialised microorganisms are equipped with the necessary enzymes (Santos and Linardi, 2001). Another example is the oxidation of $H_2$ stemming from water/sediment interactions, to methane (Kotelnikova and Pedersen, 1998). In this second example, $H_2$ as the electron donor would be depicted by substrate F in Figure2f, but would derive from sediments, whereas $CO_2$ would correspond to the electron acceptor substrate E in Figure 2f, but would not derive from minerals, but from the solution. Where no easier degradable substances are available, and since this reaction is energetically costly, individual growth of the respective microbes, and growth of colonies, are slow and sparse (right hand side of the μm scale in Figure 2f). Therefore, only low numbers of protozoa and metazoa (e.g. nematodes) are supported by this sparse producer growth (Figure 2d and e).

In the situation of an easily degradable substrate (Figure 2a-c), on the same spatial scales, there are faster reactions on the cell level (0.001 mm), higher numbers of more active microorganisms (bacteria, archaea, fungi, protozoa) on the scale of 0.01 to 0.1 mm, as well as higher numbers of grazing metazoa leading to more and faster bioturbation (see below) on the millimetre to centimetre scale, than with a refractory substrate. Note that the two situations exemplified may be situated in close vicinity both spatially and temporally (compare also Kotelnikova, 2002), e.g. because gas bubbles developing in a fast-growing situation block the exchange in the neighbourhood and create a slow-growing cut-off situation. Residence time of water and solutes will be low in those situations depicted in Figure 2a-c and high in Figure 2d-f. This micro scale variability is mixed up in conventional sampling (see also section 4.2).

The two situations depicted in Figure 2 do not necessarily occur in different zones of the aquifer but might occur at the same spot, in succession. E.g. as soon as even a micro plume of easily degradable substrate finds its way to the exact same spot where a degrader had been dormant due to lack in resources, the degrader will reactivate, grow and reproduce fast and thus build the base for a complex thriving food web as depicted in Figure 2a-c. The delay with which a dormant cell receives the signal about its preferred food source and reacts and grows into measurable communities is probably one of the reasons why bioaugmentation sometimes does not lead to the expected results.

One outcome of the variability of resources and degraders in time and space is the development of (measurable) diversity in functions, not shown in Figure 2. According to the paradigm by Baas Becking (1934) the diversity is present. However, those cells not meeting conditions that they can thrive in will be dormant and will not reproduce. For this reason, they might escape discovery from grazers and predators in contrast to the frequent and active cells and taxa. The patchily occurring growth as sketched in Figure 2a-c might create new environments, such as the cut-off produced by a gas bubble (right hand side in Figure 1), where microbial action reduces the redox

potential faster than the micro site can be replenished with electron acceptors. This may lead to cells becoming active and functional that had been dormant and would not have been predicted to be active according to meso or macro scale conditions.

The situation in Figure 2d-f might also predominate when physiological competences and/or reaction partners are still lacking which would be required for degrading a refractory food source, such as products which might be left over after fast degradation of easily degradable substances. In this (*interim*) situation, a high proportion of microbial cells not equipped to tackle the current or previous situation can be expected to be dormant, waiting for conditions to improve.

On the other hand, wherever easily degradable substances such as acetate are introduced, either by transport from the surface or as products from degradation of other compounds, fast growth of individual microbial cells and, thus, fast growth of colonies is possible and situations resemble more that at the left side of Figure 2. Protozoa and metazoa feed on these increased resources, and are a corner stone of a complex and productive food web (Figure 2a and d).

However, due to fast growth on easily degradable resources, the relevant reaction partners for the degradation reaction (e.g. dissolved oxygen), might become depleted fast, particularly in zones of the aquifer that are more or less cut-off from fast replenishment (e.g. dead-end pores in Figure 1). This newly created environment might then promote biochemical (e.g. low redox potential) reactions that were not thermodynamically likely prior to the resource depletion and might thus lead to a considerably increased (diversity of) productivity, resources, and products.

For a number of degradation pathways, two (or more) microbial taxa cooperate syntrophically (many examples are given in Timmis (2010), with one organism providing a substrate that the other organism requires for continuing the degradation, while both taxa by themselves are not able to grow on the substrate alone. Such syntrophy might add on to the degradation costs because, for the same degradation step, two organisms with their metabolism costs are involved. The overall microbial cell growth might be less and larger, more resource-demanding metazoa may not be supported and the food web (Figure 2a and d) will be simple. Neither produced gas bubbles nor colonies block the micro pores, but there is also no bioturbation (see below), thus, no "macro"pores are formed (more even distribution of pores in Figure 2e). Flow patterns in such a case thus depend largely on the grain size distribution of the material and can be expected to be much less diverse than where pore blockage and bioturbation shape the environment (see below). However, increased flow pattern diversity on the micro scale has been shown to lead to increased degrader activities (Bauer et al., 2009), and thus higher degradation. These two exemplary situations are not stable in time and space – as soon as all the electron acceptors are used up in a micro zone of easily degradable substrates (Figure 2a–c), and as soon as the environment's redox potential is subsequently reduced, the situation will change to something as depicted in Figure 2d–f). This means that in parallel to the direct toxic effects of contaminants on metazoa (Avramov et al., 2013), the contaminant type also has indirect effects on metazoa via the food web (see also section 3.2). The same is probably true for protozoa.

The lower the prevalence of bacterial, fungal, and archaea growth, the more space grazing metazoa and protozoa need to cover in order to find required food resources. They will not build large associations but will spread out to exploit the scarce environment efficiently. They will integrate over the smallest scale thus 'bridging' from the smallest to a larger scale.

The burrowing activity of the higher and larger organisms may lead to bioturbation of the matrix where sediments are soft enough (Griebler et al., 2014), i.e. particularly in shallow unconsolidated sediments, which means higher pore width heterogeneity, leading to increased exchange via the larger pores. Metazoa have indeed been found in sediments the pore size distribution of which would indicate that pores should be too small for metazoa to fit through (e.g. Schmidt et al., 2007). The fact that metazoa occur nonetheless indicates that such sediments are characterized by a secondary porosity which cannot be inferred from the grain size distribution. More sophisticated methods such as axial tomodensitometry can show the actual geometry of pore size distributions (Dufour et al., 2005; Mermillod-Blondin et al., 2003). Bioturbation and the subsequent mixing may initially lead to chemically more homogeneous conditions and less prominent gradients, but these factors will probably not outweigh the increased diversity of productivity. While some pores are widened by the perturbation, others inevitably at the same time decrease in size, and might then easily be blocked from exchange. Bioturbation was shown to enhance microbial activity in lake sediments (Baranov et al., 2016) and the same may be true for unconsolidated sediment groundwater aquifers. Thus, bioturbation (Figure 2a), based on sufficient microbial growth (Figure 2c) leads to increased spatial and temporal heterogeneity as sketched in Figure 2b and a. After all, in an environment as sparsely populated as groundwater, any biologically mediated mixing would never be complete. Total flux of water and matter at a higher scale do not change necessarily; they are largely externally controlled. However, the increased temporal and spatial diversity of habitat leads to an increased diversity of redox situations and thus to an increased diversity of processes and products. Due to increased mixing, reaction times are expected to decrease, again resulting in an increase in productivity.

Where a larger scale influence such as from contamination leads to increased diversity of productivity (see above), higher organisms might indirectly profit from products of the newly possible reactions. Alternatively, such products might be harmful and organisms will avoid those sites. Another possibility is that higher, and larger, organisms reconnect such cut-off pores due to their burrowing activities and will thus influence overall availability of resources for (biologically mediated) reactions. These considerations may have considerable impact on expected outcomes for micro scale modelling as outlined in section 4.3.

## 3.2 Significance of the interactions and processes on the micro scale for groundwater contamination

The two contrasting situations in Figure 2 (a-c versus d-f) stand for two exemplary variably-fed aquifer situations. This might be due to different exchange with the surface, with higher exchange leading to faster growth, represented by Figure 2a-c. However, exchange with the surface might also mean risk of contamination (Foster

and Chilton, 2003). Two major situations then may occur: on the one hand, contamination might consist of the input of an easily degradable, nutrient-rich substrate, such as sewage (Sinton, 1984), and microbial growth might increase, so that the situation might move even further to the left along the time axis (x axis in Figure 2), and to even faster processes. On the other hand, the contaminant might be toxic and reduce growth.

In almost all chemical reactions, one substrate acts as electron donor and one as electron acceptor. The degradation of a substrate requires usually a reaction partner. A contaminant might be an electron donor or an electron acceptor, depending on the substance, the reaction partner and the redox situation. E.g. the aerobic degradation of benzene (e.g. substrate B in Figure 2c), acting as an electron donor, requires an electron acceptor, e.g. $O_2$ or $NO_3^-$ (substrate A in Figure 2c). In reductive chlorination e.g., however, the contaminant acts as the electron acceptor, e.g. chlorinated solvents (taking up the position of substrate F in Figure 2f), and this anaerobic reaction requires an electron donor, e.g. acetate (substrate E in Figure 2f). Depending on the viewpoint and the concentrations, all mentioned substances might be considered contaminants. In the situation of an easily degradable substrate, the requirement for reaction partners might exceed the hydraulic exchange capacity, and the lack of reaction partners might slow down processes and move the situation to the right along the time axis of Figure 2. This slowing down of processes might also arise when the contaminant is toxic at least to the majority of organisms such that only specialists, requiring special resources, are able to withstand or even use the contamination. This usually comes at a high metabolic cost, which slows down processes and moves the picture even further to the right along the time (x) axis in Figure 2.

On the micro scale, due to the interplay between diffusion, advection, and sorption (compare with Figure 1 and section 2 and 3), the concentration neither of toxic substances nor of oxygen and nutrients will be the same throughout the contaminated area. Degradation might (at first) be limited to a few pores, due to either the patchy and sparse occurrence of resources and reaction partners, and/or due to the occurrence of organisms able to deal with the substrate (compare with section 3). However, this is a question of scale, as discussed directly below. As shown in the section 3, the micro scale behaviour of microbial communities can eventually have a very significant effect on the macro scale hydraulic behaviour (e.g. Tang et al., 2015).

A common paradigm, alluded to before, claims that bacteria, archaea, and fungi can disperse in an unhindered manner, with the environment determining whether single cells grow into colonies (Baas Becking, 1934). However, microbial biomass is low in groundwater and, thus, not every taxon and every biochemical reactivity is present on the smallest scale neither in time nor space. Due to dispersal, it is a matter of time until the first degrader cell harbouring the necessary physiological tools meets the contaminant, and a matter of more time until this one cell has grown into a colony which ultimately, through ecosystem services, leads to lasting changes in the contaminated environment. However, since this first encounter of the first degrader cell and the contaminant under the right circumstances occurs on the micro scale, it is important to consider the spatial and temporal distance between (contaminant) substrate and cell on the micro scale. In groundwater, this is probably only little alleviated by the nitrate, sulphur, and calcite storage capability, in conjunction with gliding motility of some bacteria (Burgin and Hamilton, 2007).

Where resources are high enough for larger and more frequent colonies of microbes to develop (e.g. in moderately concentrated easily degradable contaminant plumes, and especially in the fringe zones of plumes) microbes might use up the available electron acceptors such as oxygen so that the respective levels become critically low for animals, and the lack of oxygen might become lethal – although groundwater metazoa often still thrive at oxygen levels as low as 1 mg $L^{-1}$ (Hervant et al., 1999). While groundwater protozoa are well known to thrive under microaerobic conditions (Fusconi et al., 1999), metazoa have to find a compromise between grazing dense microbial growth and spending too much time in a detrimental environment. However, lake water fleas have been observed to "dive" for short periods into water layers which would be lethal over time because of low concentrations of dissolved oxygen and elevated concentrations of hydrogen sulphide, in order to reach better food resources (Sell, 1998) and marine sediment metazoa are known to migrate between oxic and anoxic layers (Braeckman et al., 2013). There is no ultimate reason why groundwater metazoa might not use similar strategies, of course not in a free swim, but using unfavourable or even toxic zones briefly in search for food, and then recovering to more favourable zones. The few groundwater taxa used in ecotoxicological studies so far have proven to show a different pattern to that of their surface relatives (Avramov et al., 2013; Cifoni et al., 2017; Di Lorenzo et al., 2016; Hose et al., 2016; Mösslacher, 2000), being more sensitive towards stressors than their surface relatives, or less sensitive. Thus, metazoa very much depend on what is happening on the micro scale.

Also, the contamination itself changes through time, e.g. due to degradation and adsorption. There are cases where degradation itself leads to (more) toxic compounds, such as tetrachloroethylene (PCE) which is degraded to the moderately toxic trichloroethylene (TCE) which in turn is then degraded to virtually non-degradable and very toxic vinyl chloride (Begley et al., 2012). In such a case, the subsurface community might change within the life span of a contamination, from a left-hand situation in Figure 2 to a right-hand situation where few organisms are able to thrive and be productive, and exchange with better-fed situations is not sufficient to provide the missing electron donors and/or acceptors in manageable time-spans. However, once adequate conditions are established, e.g. by engineering measures such as biostimulation and/or bioaugmentation (see section 4.1), the situation might change again to a rather well-fed, largely non-toxic, productive situation, rather such as Figure 2a-c. The contaminant may also change its physical state over time. E.g. the contaminant may be immobilized by adsorption (Canavan et al., 2006) to organic matter (including EPS; Singh et al., 2006), precipitation of metal ions (e.g., due to a change in pH) or formation of metal complexes (Watson et al., 2005). Where the change in redox state was caused by microbial actions, this means that in parallel to the direct mechanism of degradation, the indirect depletion of contaminant from the solute phase is part of microbially induced bioremediation.

Although difficult to measure, ignoring the effects of micro scale heterogeneity on hydrogeological, physical, and geochemical processes means making false assumptions on where and how biodegradation occurs and is most effective. Diffusion into micro pores that are not even reachable for microbes leads to significant delay in biodegradation in aquifers with otherwise good exchange between reaction partners and enzymes and it has been shown that diffusion may even be the major transport

mechanism (e.g. Johnson et al., 1989). But even in those pores where microbes, contaminant, and reaction partner(s) are present, the type of microbial distribution limits the biodegradation of groundwater contaminants (Heße et al., 2009; Meckenstock et al., 2014; Richnow et al., 2003). Biodegradation is clearly influenced by pore-scale processes ( Scheibe et al., 2015b; Yang et al., 2016).

## 4  Practical applications of this discussion

Groundwater management has – for want of other methods – been applied on the metre scale which may seem minute compared to the extent of the aquifer (and some of the contaminations) but which ignores the pores (Boulton et al., 2010). By how much might we be able to increase biodegradation by steering the trophic web? Micro scale sampling will probably never become routine, or subject to legislation. However, in order to make appropriately informed macro scale decisions, a better understanding of the micro scale's influence at larger scales is important. By some carefully designed research initiatives and case studies, we hope the research community will be able to identify framework conditions for which certain types of micro scale situations may offer ecosystem services which can even be triggered by macro scale management. I.e. if macro scale management introduces heterogeneity, by e.g. bioaugmenting and biostimulating with complex mixtures of substrates and organisms, instead of just one substrate and one degrader, then this will automatically increase micro scale heterogeneity that leads to spatially complex processes as a desired result and will provide niches for further organisms.

Even though first attempts have been made at coupling micro scale evaluations to catchment scale estimations (Battiato et al., 2011; Scheibe et al., 2015a), these attempts are restricted to bacterial microorganisms so far (see also section 4.3). However, it has been known for decades that protozoan grazing either reduces (Kota et al., 1999) or advances (Mattison et al., 2005) bacterial biodegradation, even under groundwater conditions. Therefore, not only is it important to include this protozoan grazing into current groundwater field studies and modelling frameworks, in order to find the conditions under which such reduction or advancement occurs. As shown in section 3.2, it may be as important, if not more important, to use not only protozoa but also metazoa in groundwater management. To this date, unfortunately, it is not possible to make assumptions on how much fauna contributes to overall degradation, because feeding, grazing, and degradation rates have rarely been measured for groundwater fauna (Di Lorenzo et al., 2016; Hervant et al., 1997; Wilhelm et al., 2006).

### 4.1 Bioremediation and the role of the micro scale

Bioremediating measures can be largely divided into biostimulation and bioaugmentation (Spira and Edwards, 2006). When living conditions on the micro scale are improved artificially, e.g. by engineering gas and organic substance exchange (biostimulation), or by inserting "trigger cells" as starting points for bacterial communities (bioaugmentation), the right hand situation in Figure 2 might change to one with faster growth, faster degradation of contamination, and sustaining either more complex food webs, or reduced food webs, depending on the outcome of

competition for resources, and might thus move to the left on the time scale (x axis) in Figure 2.

In an environment where densities of microbial cells supported are as low as they are in groundwater (pristine groundwater: usually $10^2$ to $10^6$ cells $cm^{-3}$; pristine aquifer matrix: usually $10^4$ to $10^8$ cells attached $cm^{-3}$; compiled in Griebler and Lueders, 2009), and where the majority of cells is dormant (e.g. Egli, 2010; Fredrickson et al., 1995), the insertion of a microbial start population cannot be expected to thrive immediately. The start population grows all the faster, the more appropriate the conditions (redox state; reaction partners) are, and the better the organisms are adjusted. Adjustment might be enhanced by biostimulating measures. Such biostimulating methods have been reviewed in Scow and Hicks (2005) and might include the addition of electron acceptors such as nutrients, oxygen sparging, i.e. the pumping of oxygen into the subsurface, recirculation of groundwater from the extraction well into injection wells.

Neither biostimulation nor bioaugmentation are automatically successful on their own – if there are no start populations within the temporal and spatial scale of observation, then there are no organisms able to use artificially elevated concentrations of electron acceptors and donors – or concentrations are too high, stressing the organisms. On the other hand, just adding cells without making sure that these cells meet the necessary resources before becoming prey, is equally doomed to fail (probably the explanation for the failure of bioaugmentation in e.g. Bouchez et al., 2000). Both resources and biomass need to be finely tuned (Meckenstock et al., 2015), especially for potential micro scale situations, and need to be adjusted to the respective background hydraulic and geological site characteristics. More research is necessary into this fine interplay, and into the circumstances under which opportunities for future development may become usable in the future, which have gone undiscovered so far, but it stands to reason that micro scale patterns are pivotal.

Where fauna are not excluded by a contaminant, they might be managed to increase microbial contaminant degradation by rejuvenating the biofilm, as shown for some protozoa (e.g. Mattison et al., 2005). No peer-reviewed research has yet been conducted into such engineering by adjusting faunal assemblages in the field. There is a scientific gap that needs to be bridged by further research, but for now we hypothesise that fauna can potentially be used as a biomanipulation tool.

## 4.2   Sampling micro scale variability

Sampling at the commonly applied scale, via groundwater wells, even if spatially highly resolved as in Anneser et al. (2008), will miss the fundamentally different situations on the smaller scales as presented in Figure 1 and Figure 2. The scale of samples (often not below 1 decimetre/ 1 Litre) in relation to the scale at which the most influential patterns occur, will determine whether correlations between physical and chemical variables are meaningful or arbitrary. This is exacerbated for correlations with organisms: microorganisms of below 1 µm size using micro patches and forming micro colonies (< 100 cells) "see" an environment which is up to two orders of magnitude smaller than the sample taken routinely. Assuming that due to microbial action there is a strong gradient in solutes in the micro metre environment of such colonies, a 1 L sample is always a mixture. This mixed sample cannot be

expected to correlate with physical and chemical factors measured at and thus averaged over, the centimetre or decimetre scale. The consequence is that much of the autecological knowledge on groundwater metazoa, stemming from such correlations from field investigations, is at best misleading.

In addition, plant communities have been shown to be more dependent on the multitude of factors than on the minimally restricting factor (Liebig's law) recently (Harpole et al., 2016). With groundwater organisms as well, it is likely that the interactions of factors is more important than the concentration of a single constituent (e.g. Schmidt et al., 2007). Controlled experiments would be enormous. This is where numerical simulations can become an option to test hypotheses (see section 4.3).

In order to fully understand the degradative opportunities that an aquifer offers, sampling needs to respect situations on the smallest scales because such situations may be mutually exclusive and thus cannot be approximated by scaling up and combining processes on a larger scale (Anneser et al., 2008). Sampling at scales too large may lead to paradox patterns such as overlapping of both electron acceptors and electron donors without reaction, as detailed in Bauer et al. (2008) or Bertrand et al. (2016). These authors conclude that there must be one or more additional factors limiting biodegradation. Such a factor might be the availability of resources in micro pores, as shown conceptually above, which is still at a scale too small to be measured even with the sophisticated setup of Bauer et al. (2008).

Like with bacteria, archaea and protozoa, sampling of metazoa as well might focus on the sediment or the free water. Conventionally, small benthic organisms from marine sediments are enumerated by elutriation or a Ludox centrifugation technique (Du et al., 2009). Elutriation can only be applied to medium and coarse sands. Ludox centrifugation has been verified as the more efficient technique for fine to silty sediments (Du et al., 2009). However, densities of metazoa in groundwater are so low and patchy that considerable amounts of sediment would have to be brought to the surface in order to apply these methods. One-off samples have provided valuable input at least for microbes, but have – to the authors' knowledge – never been applied to metazoa. Regular, e.g. monthly, monitoring by extracting the then necessary volumes of solids, i.e. cubic meters, from the subsurface is very expensive, the meaning of repeated samples even from closely spaced cores is fraught with difficulty, and the aquifer with its flow and exchange characteristics is heavily disturbed which will impede on subsequent sampling. Therefore, metazoa sampling usually focuses on the groundwater itself, not the sediments.

Sampling metazoan patterns on the adequate meso scale is so far only possible in stacked traps (Hahn, 2005) which are pumped. While such traps provide an artificial environment and are thus not overly representative for the real situation, they may be an adequate tool for regular monitoring, at least of shallow groundwater. Metazoa in groundwater beyond the hyporheic zone where Bou-Rouch pumps have been found to deliver meaningful samples (Boulton et al., 2003), are usually sampled by nets. Particularly for deeper groundwater, the fastest and least disturbing sampling method remains any adaptation based on Cvetkov (1968), i.e. lowering a narrow plankton net several times down into the groundwater well. But spatial resolution within a well is impossible with this method. Note also that most contributions on sampling fauna in groundwater only aim at metazoa, not protozoa at the same time.

The net sampling will unfortunately always underrepresent small-bodied metazoa and largely miss protozoa. However, densities even of the smallest metazoa and protozoa in pumped groundwater are so low that volumes that would need to be live sorted as in Gasol (1993) would be large. For the enumeration of protozoa from sandy samples a few methods have been developed in order to enrich cells, also from suspended sediments: fluorescence in situ hybridization (Diederichs et al., 2003), quantitative centrifugation (Starink et al., 1994), quantitative protargol stain (QPS) (Montagnes and Lynn, 1993), and the Ludox-QPS method (Du et al., 2009). However, all these methods require a certain density of organisms in the sediment that is not usually reached in groundwater sediments. Some method of enriching metazoa and protozoa from large water volumes is needed.

For the time being, it seems more fruitful to study biogeography particularly of those metazoa and protozoa too small to be caught by plankton nets by their molecular variation (e.g. DNA; Brad et al., 2008; Euringer and Lueders, 2008). But from the molecular signal it is impossible to derive the actual abundances and biomasses of taxa – even if a quantitative measurement of DNA is possible, it cannot be resolved from how many organisms this DNA was derived.

Another issue is that the groundwater well environment provides associations that are different to those in the surrounding groundwater in terms of metazoa (Korbel et al., 2017; Matzke and Hahn, 2005; Sorensen et al., 2013; Steenken, 1998), protozoa (Korbel et al., 2017), and bacteria (Korbel et al., 2017; Roudnew et al., 2014; Sorensen et al., 2013). The best recommendation to date is to combine methods in the least disturbing way in order to complement results.

More sophisticated methods are urgently needed (Gutjahr et al., 2013; Hancock and Boulton, 2009). Until it is possible to sample at the relevant scales, most theoretical understanding will have to come from modelling (section 4.3).

## 4.3    Modeling the micro scale

On the macro scale, groundwater flow is usually estimated using the Darcy equation. However, this equation is not applicable to the micro scale with its parabolic flow profile, described by the Hagen Poiseuille equation. Many approaches are possible, e.g. calculating micro scale flow by using computational fluid dynamic implementations that solve the parameter-intensive equations such as (simplified) laminar Navier-Stokes numerically, thus deriving near-continuous patterns, as reviewed by Xiong et al. (2016).

Two major challenges when taking into account the biological growth in groundwater pores are 1) upscaling to a scale relevant for management, and 2) the discontinuous biological distribution patterns. Micro scale models have been coupled to larger scale models by Tartakovsky et al. (2013) whose genome-scale model was used to predict biomass yield and stoichiometry for iron consumption, in comparison to prior Monod formulations based on energetics considerations", by Cuthbert et al. (2013) who upscaled microbially induced calcite precipitation, by Orgogozo et al. (2013) who developed an approach to Stokes type solute transport in an unconsolidated medium

with biofilm growth combined with a quasi-steady linear closure for the bioreactions, and by Scheibe and colleagues (Scheibe et al., 2015a, 2015b, 2015c) who developed a general framework. In Scheibe et al. (2015c) and Scheibe et al. (2015a) reactivity is modelled along interfaces. However, none of these approaches tackles the discontinuous biological growth patterns at the same time.

The discontinuous growth and behaviour of low numbers of biological individuals cannot be represented well by continuous equations or models, since individuals make seemingly arbitrary decisions, discrete in time and space (Grimm et al., 2016). With the low biotic density in groundwater, individualized approaches are more informative than calculating averages with huge error margins. To simulate discontinuous biotic growth, a number of approaches have been developed: e.g. cylindrical plates of biomass with a reactive surface but lacking individual cell properties and behaviour (Molz et al., 1986), a domain-wise localized biofilm phase that does not grow and thus allows neither for competition nor grazing (Orgogozo et al., 2013), cellular automata (Picioreanu et al., 1998) or individual-based approaches (Kreft et al., 1999; Lardon et al., 2011). Coupling microbial discontinuous growth to the continuum-scale has been done in a hybrid approach by Tang et al. (2015). However, in this hybrid approach, flow was neglected and the boundary conditions were modified so that diffusive mixing of reactants alone controlled the reaction. The simulated domain could be imagined as a balloon within a larger water body with permeable walls, but is in principle something between a chemostat and a circular boundary set-up. Both of these approaches are implemented also in iDynoMiCS (Lardon et al., 2011), but neither allows for advective flow, nor does iDynoMiCS in its present form. In addition, the approach by Tang et al. (2015) does not allow for multispecies interactions within a food web in flowing groundwater. Picioreanu et al. have coupled individual-based models that are less complex than iDynoMiCS, with advection (e.g. Picioreanu et al., 2010, 2000, Radu et al., 2015, 2010), but they have not implemented food webs. For further details on how these approaches work, what their advantages are and what they permit to highlight, the reader is referred to the extensive review by Xiong et al. (2016) which cannot be repeated here in a few lines in the framework of this discussion.

However, none of those approaches includes trophic networks, i.e. grazing, predation, etc. This means that until now, not only are there few investigations on the interactions of organisms on different scales under different nutritional situations neither *in situ* nor *in vitro* (see previous sections, particularly section 4.2), but also the frameworks for evaluating possible situations computationally are still only developing (Schmidt et al., 2011). Currently, a framework based on a set up similar to the one in Picioreanu et al. (2010) coupling a multiphysics solver with an individual-based model is validated against the published 1D approach by Heße et al. (2010, 2009) and is already able to demonstrate how relevant microbial distribution patterns at the micro scale are for *in situ* biodegradation rates. In particular, the micro scale aggregation of bacterial cells into colonies is shown to lead to a severe restriction of the bioavailability of the substrate and to an associated reduction of the effective degradation rate also in cases where a homogeneous distribution of cells along the pore wall would not lead to major restrictions of bioavailability. This framework thus allows for more realistic scenarios and will be extended to allow for food web interactions and also for e.g. plasmid transfers such as in Merkey et al. (2011). It is well known that degradation of e.g. toluene depends on the transfer of the TOL

plasmid (de Lorenzo, 2008; Williams and Worsey, 1976). How this plasmid is transferred within the groundwater microbial community as part of the food web has not been investigated yet, neither in vivo, nor in vitro, nor in silico.

Quantitative knowledge on faunal rates is urgently needed for food web models. For the time being, models will be based on assumptions founded on educated guesses. Improved models can be used to test how important interactions between processes operating at different scales actually are for a range of different hydrogeological contexts. Such models enable evaluating remediation measures on different scales for contaminated groundwater systems. Three questions that will rather be tackled by modelling than by sampling in the foreseeable future are 1) what is the minimum resource (in the form of carbon and nutrients; but also radiation; Chivian et al., 2008) requirements for a system to support which level of food web complexity? 2) Which processes, and, in consequence, which ecosystem services are supported by which level of resources? 3) To which extent will micro scale ecosystems impact the groundwater system?

### Conclusions

Ultimately, it is necessary to make aquifer-wide assumptions on processes, functionality, and sheer numbers of microorganisms and fauna, when addressing issues of groundwater quality management. In our opinion, and as we have outlined here, this will only be possible by more rigorously understanding the micro scale through improved field and laboratory investigation and modelling approaches, and then integrating this new knowledge within emerging understandings of meso and macro scale processes – these processes at all the different scales interact with each other so that they have to be considered together.

In particular, we need to know what the ranges are for organism abundances and functional rates, particularly regarding fauna, which act on their immediate surroundings but thus impact larger scales as well. We need to define the range of conditions under which different types of groundwater ecosystem functions are performed in order to define, for example, the circumstances in which dormant organisms and their respective functionality can be reactivated. Once this is known, engineering concepts can be developed addressing how even dormant microbial cells which are located in micro pores and which cannot easily be reached by flow nor biostimulation, can be triggered to participate in organic contaminant degradation. This will only work if faunal grazing and predation is steered towards rejuvenating microbial growth instead of diminishing it.

It may be a long time before all the necessary relevant field and laboratory investigations have been carried out to develop adequate engineering strategies. In the meantime, modelling the trophic network on all scales in all types of groundwater aquifers (not just well-studied highly porous granular aquifers, but also hard-rock and highly cemented and/or fractured sedimentary aquifers) is urgently needed in order to inform the design of such field and laboratory research. As sampling the micro scale eventually improves the data with which to validate new models, the model-based and

field-based approaches can then iteratively inform each other's experimental/modelling design to make real progress in understanding the groundwater micro scale ecosystem dynamics.

The need for an integrated understanding of the micro scale, for better understanding ecosystem functions on all scales, is therefore both good and bad news. The bad news is that we do not know how to sample or model appropriately, and a large research effort is first needed to develop better techniques and then to observe, understand and model the micro scale adequately. The good news is that, as our understanding increases, we are sure to better find ways of employing a vast army of 'micro' helpers to support our groundwater management if only we learn how to help them by providing them with the appropriate micro scale living conditions.

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Yang, X., Mehmani, Y., Perkins, W. a., Pasquali, A., Schönherr, M., Kim, K., Perego, M., Parks, M.L., Trask, N., Balhoff, M.T., Richmond, M.C., Geier, M., Krafczyk, M., Luo, L.-S., Tartakovsky, A.M., Scheibe, T.D., 2016. Intercomparison of 3D pore-scale flow and solute transport simulation methods. Adv. Water Resour. 95, 176–189. doi:10.1016/j.advwatres.2015.09.015
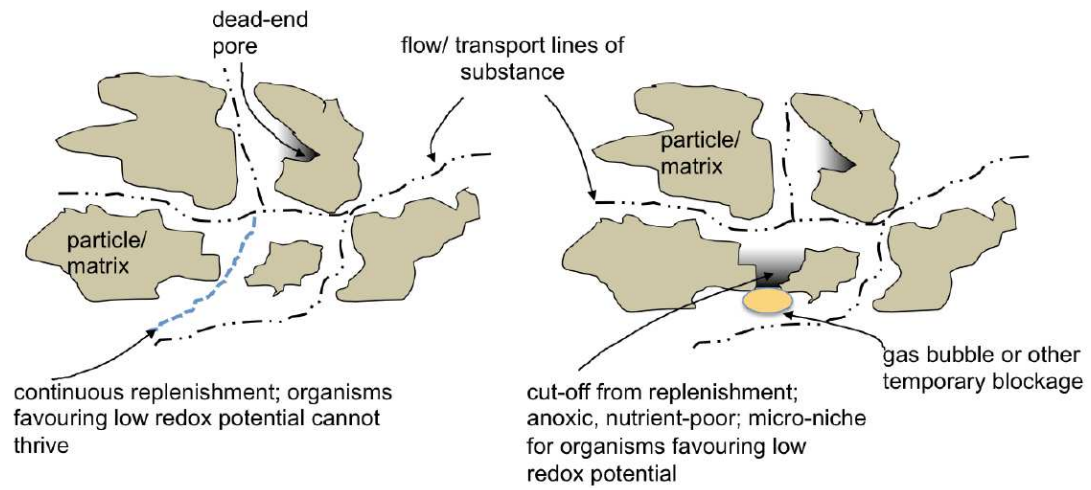
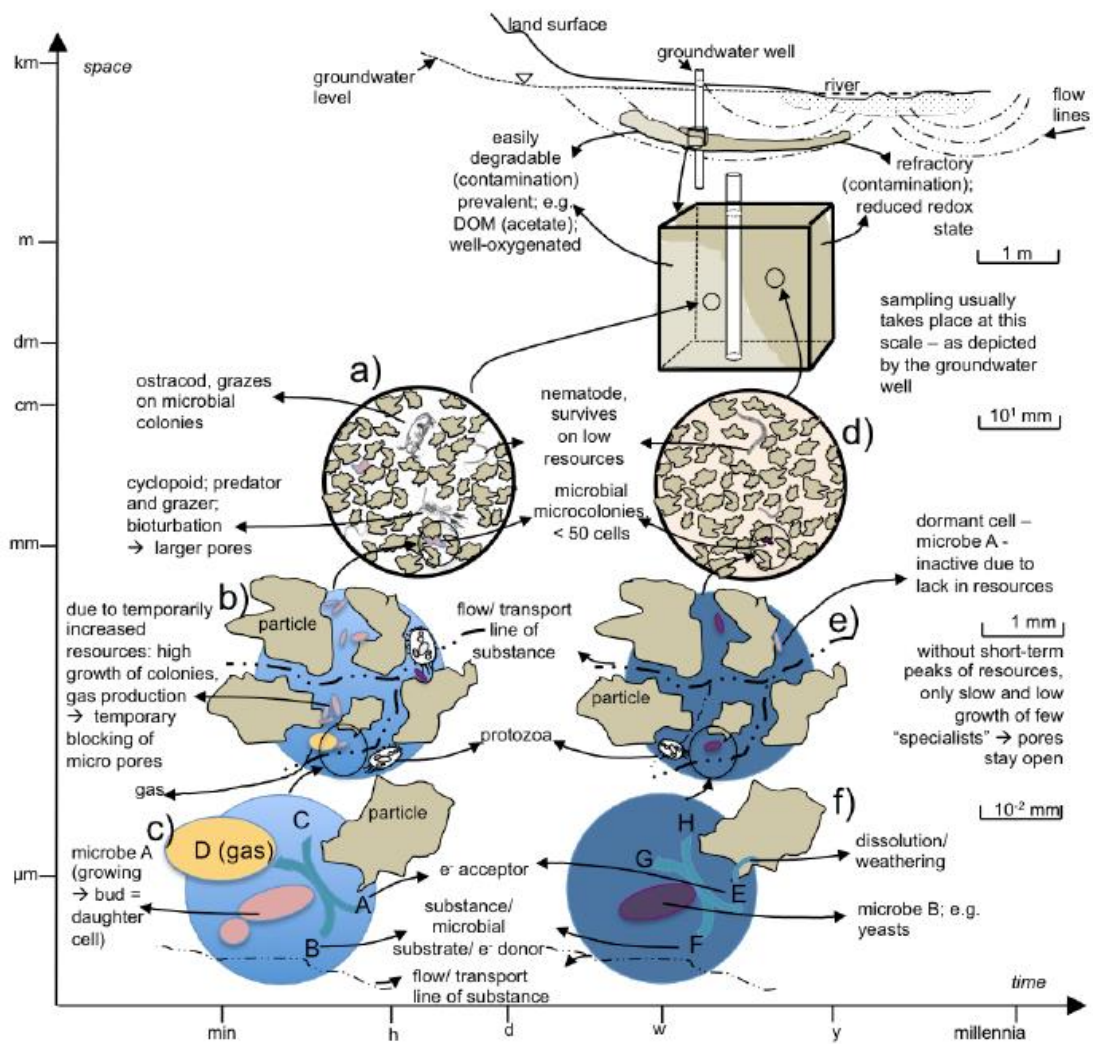Figure 1: Conceptual sketch of the micro scale variability in pores and their hydraulic situation.

Figure 2: How micro scale features translate to patterns on larger scales, illustrated using examples of two different zones that might be different stages of a contamination being broken down, or which might represent recharge/background with carbon compounds of different complexity. Temporal scale on the x-axis; spatial scale on the y-axis. Axes should indicate roughly the different dimensions on which the patterns appear, but the figure as such is not to scale.